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Abstract

Pectinophiles are bacteria that utilize pectin and only a few related compounds as substrates. Obligately anaerobic pectinophiles have been isolated from the intestinal tracts and gingivae of humans and from the rumina of cattle. We isolated three strains of pectinophilic bacteria from colonic contents of pigs but were unable to isolate pectinophiles from the rumen contents of four sheep, even when the animals were fed a high-pectin diet. The pectinophiles isolated from pigs were strictly anaerobic, motile, gram-positive rods (0.36 to 0.56 by 2.4 to 3.1 μm). Pectin, polygalacturonic acid, and gluconate were the only substrates that supported rapid growth. All three strains grew slowly on either lactose or cellobiose and fermented fructose after a lag of several days. Pectin was degraded by means of an extracellular pectin methylesterase and a Ca^{2+} -dependent exopectate lyase. A comparison of the 16S rRNA sequences of these isolates with the 16S rRNA sequences of other gram-positive bacteria revealed a specific relationship with *Lachnospira multipara* (level of similarity, 94%). The Gram reaction, formation of spore-like structures, and the utilization of lactose and cellobiose differentiated the pig isolates from previously described pectinophiles. The pig isolates represent a previously undescribed species of the genus *Lachnospira*, for which we propose the name *Lachnospira pectinoschiza*.

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Comments

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Lachnospira pectinoschiza sp. nov., an Anaerobic Pectinophile from the Pig Intestine

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Pectinophiles are bacteria that utilize pectin and only a few related compounds as substrates. Obligately anaerobic pectinophiles have been isolated from the intestinal tracts and gingivae of humans and from the rumina of cattle. We isolated three strains of pectinophilic bacteria from colonic contents of pigs but were unable to isolate pectinophiles from the rumen contents of four sheep, even when the animals were fed a high-pectin diet. The pectinophiles isolated from pigs were strictly anaerobic, motile, gram-positive rods (0.36 to 0.56 by 2.4 to 3.1 μ m). Pectin, polygalacturonic acid, and gluconate were the only substrates that supported rapid growth. All three strains grew slowly on either lactose or cellobiose and fermented fructose after a lag of several days. Pectin was degraded by means of an extracellular pectin methylesterase and a Ca^{2+} -dependent exopectate lyase. A comparison of the 16S rRNA sequences of these isolates with the 16S rRNA sequences of other gram-positive bacteria revealed a specific relationship with *Lachnospira multipara* (level of similarity, 94%). The Gram reaction, formation of spore-like structures, and the utilization of lactose and cellobiose differentiated the pig isolates from previously described pectinophiles. The pig isolates represent a previously undescribed species of the genus *Lachnospira*, for which we propose the name *Lachnospira pectinoschiza*.

Most pectin-degrading bacteria in gastrointestinal habitats are able to utilize a diversity of substrates. However, several bacteria isolated from human intestinal tracts (15) and gingivae (34, 38) and one spirochete isolated from a bovine rumen (41) are pectinophiles since they ferment only pectin and a few related compounds. We hypothesized that other pectinophiles may be present in gastrointestinal habitats but have not been isolated because pectin generally has not been included in the complex media used to enumerate microbial populations. In this paper we describe our search for rumen pectinophiles and characterize pectinophiles that we isolated from the large intestines of swine.

(A preliminary report of this work has been presented previously [7a]).

MATERIALS AND METHODS

Samples from animals. Four sheep (weight, 27 to 76 kg) were fed a diet consisting of alfalfa (ad libitum) and Sheep Feed 590 (Ralston Purina, St. Louis, Mo.) (900 g per animal per day). After 1 month the diet fed to two animals was changed to sugar beet pulp (American Sugar Co., Moorehead, Minn.) (600 g per animal per day) and wheat straw (ad libitum). Following a 2-week stabilization period for each diet, rumen fluid was collected through a rumen cannula approximately 4 h after feeding.

Five pigs (weight 15 to 30 kg) were fed a diet consisting of corn (65%) and soybean meal (30%). After 2 to 4 weeks, the animals were sacrificed and the cecal contents were collected.

Samples were processed within 0.5 h of collection. Whole samples were homogenized with a Waring blender under a CO_2 atmosphere for 1 min. An 11-g portion was mixed with 99 ml of anaerobic dilution solution (andil) (5) and homoge-

nized under a CO_2 atmosphere for an additional 1 min, and serial 10-fold dilutions in andil were prepared.

Media and physiological tests. Medium 134-11 contained (per liter) 360 ml of energy-depleted rumen fluid (2), 1.6 g of K_2HPO_4 , 1.6 g of KH_2PO_4 , 3.2 g of $(\text{NH}_4)_2\text{SO}_4$, 3.2 g of NaCl, 0.3 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.15 g of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1.0 g of cysteine HCl \cdot H_2O , 0.001 g of resazurin, 4.0 g of Na_2CO_3 , and 2.5 g of ethanol-washed (17) polygalacturonic acid (PGA) (Sigma Chemical Co., St. Louis, Mo.). Modified BGD medium (15) contained (per liter) 2.0 g of KH_2PO_4 , 6.0 g of K_2HPO_4 , 2.5 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.15 g of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.02 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 1.4 g of $(\text{NH}_4)_2\text{SO}_4$, 1.0 g of cysteine HCl \cdot H_2O , 0.001 g of resazurin, 4.0 g of PGA, 1.0 g of NaHCO_3 , and 2.0 g of yeast extract. Roll tubes contained 2% agar. The carbohydrates and amino acids tested as potential substrates were added as filter-sterilized solutions to a final concentration of 0.2% to medium PF base (15) without PGA. All media were prepared and dispensed anaerobically under a CO_2 (medium 134-11), N_2 - CO_2 (90:10) (medium PF), or N_2 (BGD medium) atmosphere.

Triplicate roll tubes were inoculated with 0.2-ml portions of diluted gastrointestinal contents. Total counts were determined by using medium CCA (2) and medium CCA containing 0.2% PGA. Pectin-utilizing bacteria were enumerated by using medium 134-11 and medium PF. The tubes were incubated at 39°C for 72 h. Colonies were counted, and about 20 colonies were randomly picked from medium 134-11 and medium PF preparations and inoculated into medium 134-11 broth and medium PF broth, respectively. After 24 to 48 h, the isolates were examined by using the Somogyi-Nelson assay (28) for the production of reducing sugars from PGA. Isolates which utilized glucose and/or other sugars in addition to pectin were not characterized further.

Catalase production was tested by using cells grown on medium PF agar plates in an anaerobic chamber (Coy Laboratories, Ann Arbor, Mich.) containing 5% CO_2 -10% H_2 -85% N_2 atmosphere. The plates were exposed to room atmosphere for 30 min prior to the addition of 3% H_2O_2 .

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Medium PF broth supplemented with 0.1% KNO₃ but lacking cysteine and resazurin was used to determine nitrate reduction. Medium PF broth supplemented with 12% gelatin and medium PF broth supplemented with 2% oxgall were used to test for gelatin hydrolysis and bile tolerance, respectively. Medium PF broth supplemented with 0.1% esculin was observed under long-wavelength (366-nm) UV light for fluorescence of intact esculin (11).

Microscopy. Spore formation was determined after cultures were grown on medium PF and on egg yolk agar (EYA) supplemented with 0.4% PGA. The cultures were incubated at 30 and 39°C for 2 weeks. Spore stains were prepared by the Schaeffer-Fulton method (10). Resistance to heat (13) and resistance to ethanol (18) were also determined.

Log-phase cells grown in medium PF were harvested and washed with distilled water. Negative stains were prepared with 0.5% phosphotungstic acid (35).

Cells to be sectioned for electron microscopy were grown in BGD medium (15) to log phase or on EYA slants at 30°C for 7 days. The cells were harvested and fixed for 2 h in 2.5% glutaraldehyde in 0.1 N sodium cacodylate buffer. The fixed cells were embedded in 2% agar, dehydrated in ethanol, and infiltrated with resin. Ultrathin sections (thickness, approximately 60 nm) were cut with a diamond knife, stained with 2% uranyl acetate and lead citrate, and then examined with a Philips model 410 transmission electron microscope.

Fermentation products. Butyl esters (33) of volatile fatty acids were analyzed with a gas chromatograph (model 5890; Hewlett-Packard, Avondale, Pa.) equipped with a hydrogen flame ionization detector. This instrument was also used at 40°C to detect alcohols. A series 580 gas chromatograph (Gow Mac, Bridgewater, N.J.) equipped with Pora Pak Q 80/100-mesh columns and a thermal conductivity detector was used to measure H₂ and CO₂ concentrations in headspace gases of cultures grown on modified BGD medium under an N₂ atmosphere. After acidification (0.5 ml of 6 N HCl per 20 ml), samples were removed from sealed culture tubes with a gas-tight syringe and injected into the sample loop (0.5 ml). Helium was the carrier gas.

Protein profiles. Proteins were separated by denaturing polyacrylamide gel electrophoresis (PAGE), using the discontinuous buffer system of Laemmli (19), a 4% stacking gel, and a 12% separating gel. Proteins were stained with Coomassie blue R-250.

Long-chain fatty acids. Long-chain fatty acids were extracted from whole cells grown in 300 ml of medium PF. Cell pellets were lysed at 100°C in 2.5 ml of 2 N HCl for 16 h. Chloroform (2.5 ml) was added to partition the lysates. After 1.5 ml of methanol-HCl (11:1) was added, the chloroform extracts were heated for 30 min at 55°C. The samples were washed three times with water and then evaporated to dryness under N₂ (1). The esters were resuspended in hexane and analyzed with a Hewlett-Packard model 5890 gas chromatograph. The column temperature was increased at a rate of 4°C/min between 150 and 240°C. The peaks were compared with the peaks produced by known standards, but no additional tests to positively identify individual fatty acids were performed.

G+C content of DNA. Cell pellets were lysed by the method of Hull et al. (14), and the DNA was isolated by CsCl gradient centrifugation. The G+C content was calculated by the thermal denaturation method (24), using a Gilford Response II spectrophotometer (Ciba Corning Diagnostic Corp., Oberlin, Ohio) equipped with a thermoprogrammer. *Escherichia coli* ATCC 14763 (G+C content, 51.4 mol%) was used as a control.

Pectinolytic enzymes. Extracellular enzymes in the culture supernatant were partially purified by acetone precipitation by using the procedure of Weber and Canale-Parola (38). Pectate lyases and polygalacturonases were detected by using the thiobarbituric acid assay (15) and the Somogyi-Nelson reducing sugar assay (28). Protein concentrations were measured by using a modification of the assay of Lowry et al. (31) and bovine serum albumin as the standard. Pectin methylesterase activity was assayed by the hydroxamic acid reaction (25).

The oligomers of PGA produced by the partially purified enzyme preparation were separated and identified by thin-layer chromatography (15). The reaction mixture (30 µl) was spotted onto type E-13255 cellulose plates (Eastman Kodak, Rochester, N.Y.), which were developed twice in ethyl acetate-acetic acid-water (4:2:3, vol/vol/vol) at 25°C (23). The plates were sprayed with the *p*-anisidine reagent (7) and heated at 100°C for 5 to 10 min. D-Galacturonic acid, digalacturonic acid, and trigalacturonic acid (Sigma Chemical Co.) were used as standards.

Sequencing of 16S rRNA. The almost complete 16S rRNA sequence of each isolate was determined by using RNA template sequencing. Total nucleic acid (primarily RNA) was extracted from approximately 50 to 100 mg of cells with hot phenol (30), and the nucleotide sequence was determined by the dideoxynucleotide method, using reverse transcriptase (21). Three universal sequencing primers (21) and four additional oligonucleotide primers were used to determine nearly complete 16S rRNA sequences (20, 26). Only the three universal sequencing primers were used for the characterization of *Lachnospira multipara*. In addition to the standard deoxynucleotide reaction experiments, reaction experiments in which inosine was substituted for guanosine were performed with all sequencing primers. The inclusion of inosine resolved many sequencing gel ambiguities caused by premature termination and band compression in GC-rich regions of the transcripts.

Sequence analysis. 16S rRNA sequences were aligned on the basis of conserved features of primary and secondary structures (36, 38). The sequences of strain 150-1^T (T = type strain), *L. multipara* D32 and 40, and *Roseburia cecicola* were determined in this study. Other sequences used for analysis were obtained from the RNA Database Project (29). These sequences were the sequences of *Clostridium aminovalericum* ATCC 13725, *Clostridium oroticum* ATCC 13619, *Clostridium symbiosum* ATCC 14940, *Clostridium coccooides* ATCC 29236, *Streptococcus hansenii* ATCC 27752, *Clostridium barkeri* ATCC 25849, *Clostridium pasteurianum* ATCC 6013, *Clostridium ramosum* 113-1, *Clostridium innocuum* B-3, *Lactobacillus casei* subsp. *casei* ATCC 393, and *Bacillus subtilis*. Levels of sequence similarity were calculated by using only nucleotide positions at which alignment in all of the sequences compared was unambiguous. The levels of similarity were converted to evolutionary distances by the method of Jukes and Cantor (16). A phylogenetic tree was constructed on the basis of inferred evolutionary distances by using the algorithm of DeSoete (8). In addition to the evolutionary distance method, relationships were examined by using the DNABOOT program of PHYLIP (phylogeny inference package) (12); this program implements the bootstrap method of placing confidence limits on phylogenies by using parsimony.

Nucleotide sequence accession numbers. The GenBank accession numbers for the sequences determined in this study are as follows: strain 150-1^T, L14675; *L. multipara* D32, L14674; *L. multipara* 40, L14673; and *R. cecicola*, L14676.

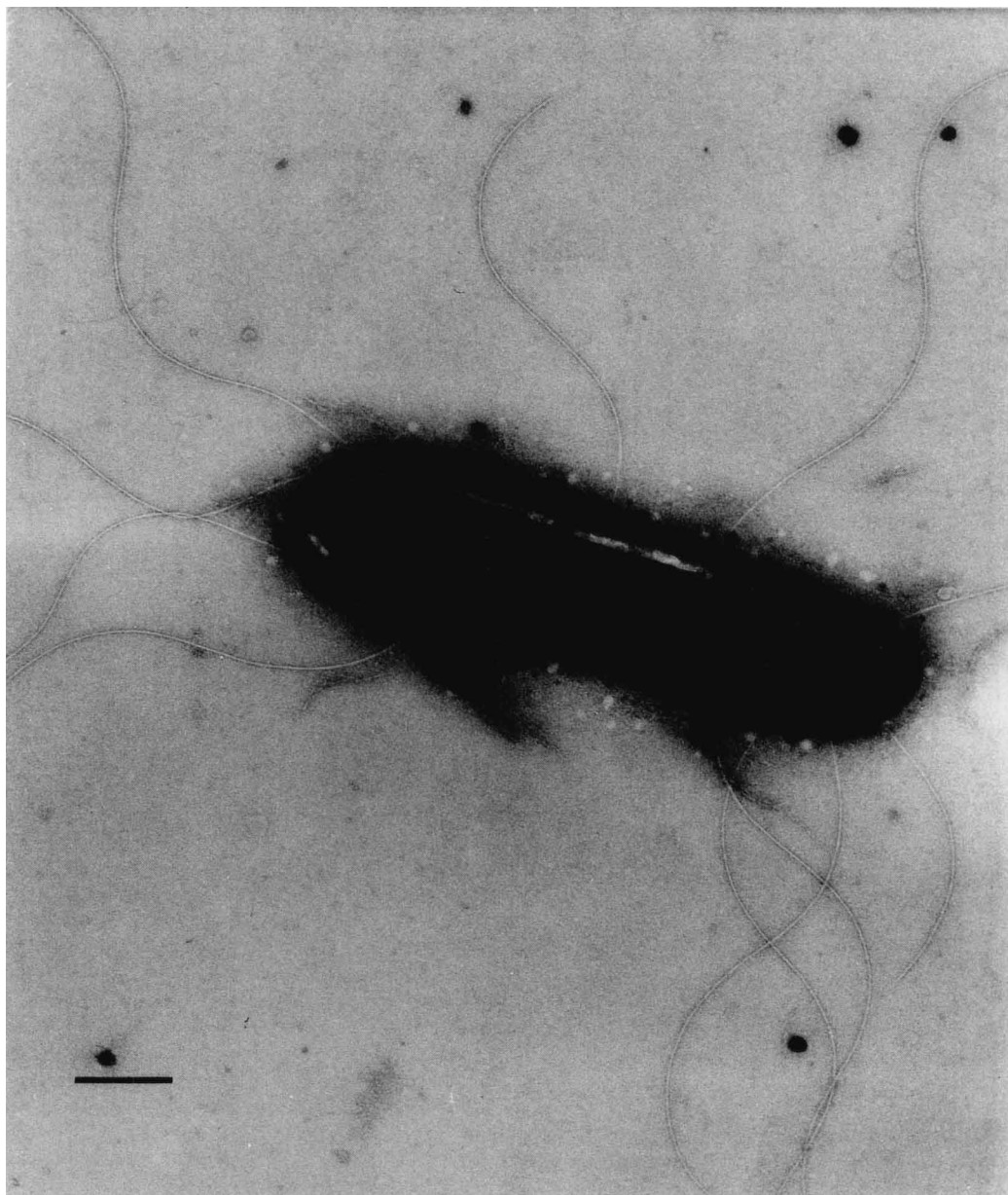


FIG. 1. Electron micrograph of a strain 131-8 cell stained with 0.5% phosphotungstic acid. The culture was grown to mid-log phase in medium PF. Bar = 0.5 μm .

RESULTS

Isolation. Six samples of rumen contents from four sheep fed an alfalfa diet were cultured for pectinophiles. Of 177 isolates screened, 66 (37%) were pectinolytic. These bacteria were not pectinophiles, however, because they utilized glucose and/or other sugars in addition to PGA. Three samples of rumen fluid from sheep fed beet pulp and wheat straw yielded a higher percentage of pectinolytic isolates (64%); however, no pectinophiles were isolated.

One fecal and four cecal samples were cultured from five pigs. Of 93 isolates tested, 12 (13%) were pectinolytic. Three additional strains, each isolated from a different animal and all isolated from roll tubes containing medium 134-11 that had been inoculated with 2×10^{-7} ml of cecal contents, were

pectinophilic. These strains were designated strains 122-19 (fecal), 131-8 (cecal), and 150-1^T (cecal).

Phenotypic description. All three pectinophilic isolates were strictly anaerobic, gram-positive rods (0.36 to 0.56 by 2.4 to 3.1 μm) that grew at temperatures ranging from 30 to 45°C. The peritrichous cells were motile by means of 6 to 18 flagella (Fig. 1). Pectin, PGA, D-gluconate, cellobiose, and lactose were fermented. D-Fructose was utilized after a lag period of 2 to 6 days. D-Galacturonic acid, the monomeric unit of PGA, was not utilized by any of the strains. Other carbohydrates and amino acids were not fermented. Growth on gluconate, pectin, and PGA was rapid; the estimated population doubling time was 55 min when PGA was the substrate. The doubling times were 4 to 6 h when fructose or lactose was used as the substrate and 7 to 10 h when

TABLE 1. End products formed by strain 150-1^T grown on different substrates

Substrate	Concn (μmol/ml of culture broth) of:			
	Formate	Acetate	Ethanol	Methanol
PGA ^a	7 ^b	9	<1	0
Pectin ^c	7	6	<1	5
Lactose	8	3	15	0
Cellobiose	7	3	12	0
Fructose	8	3	11	0

^a Strain 150-1^T was grown in modified BGD medium containing 0.2% PGA.^b Mean of three samples.^c Strain 150-1^T was grown in medium PF containing 0.2% carbohydrate.

cellobiose was used. The major end products of PGA fermentation by strain 150-1^T were formate and acetate (Table 1). Ethanol and CO₂ (2 μmol/ml) were also formed. Methanol was produced when pectin was fermented. When lactose, cellobiose, or fructose was utilized, ethanol was the most abundant product. Similar data were obtained for strains 122-19 and 131-8. Tests for catalase activity, nitrate reduction, gelatin hydrolysis, H₂ production, and esculin hydrolysis were negative. Bile did not inhibit growth. Spore-like structures were detected by light microscopy in stained cell preparations of all three strains that had been cultured for 14 days at 30°C on EYA supplemented with 0.4% PGA. Inclusions which appeared to be endospores were also observed in electron micrographs of ultrathin sections of cells grown on EYA (Fig. 2). However, no survivors were detected after the cultures had been exposed to temperatures of 60 to 80°C for 5 min or to 50% ethanol for 45 min. These

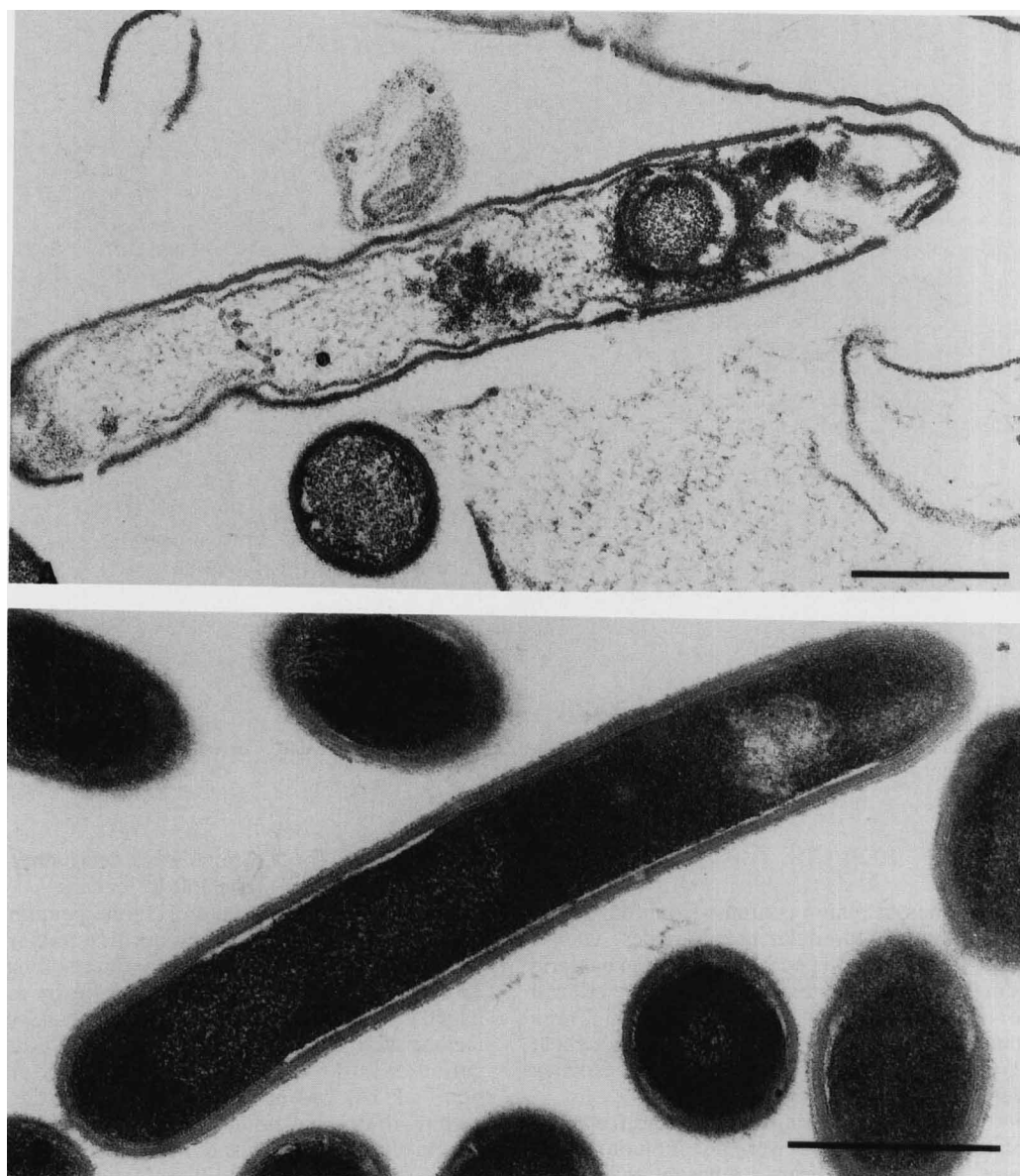


FIG. 2. (A) Electron micrograph of a thin section of a strain 122-19 culture grown on EYA for 7 days. Bar = 0.5 μm. (B) Electron micrograph of a thin section of a strain 131-8 culture grown to exponential phase in BGD medium. Bar = 0.5 μm.

TABLE 2. Specific activities of extracellular pectate lyase with and without EDTA and CaCl₂

Substrate prepn	Sp act (U min ⁻¹ mg of protein ⁻¹) ^a		
	Strain 122-19	Strain 131-8	Strain 150-1 ^T
PGA	2.7	3.2	2.4
PGA + 0.5 mM EDTA	0.2	0.3	0.2
PGA + 0.5 mM EDTA + 1 mM CaCl ₂	2.5	3.6	2.6
Pectin ^b	0.8	1.3	1.4

^a One unit was defined as the amount of enzyme that resulted in an increase in *A*₅₄₈ 1.0 in the thiobarbituric acid assay (15).

^b Pectin was substituted for PGA in the reaction mixture.

structures were not observed in cells grown on medium PF under similar conditions. The sodium dodecyl sulfate-PAGE profiles of total cell proteins from the three isolates were similar. The profiles of methyl esters of long-chain fatty acids extracted from whole cells were also similar, although the quantities of individual acids varied among strains. A fatty acid with the same retention time as palmitic acid (16:0) was the predominant fatty acid present. A second peak correlated with the retention time of myristic acid (14:0). The average G+C contents of DNA preparations, from two different determinations, were 42 mol% for strains 122-19 and 150-1^T, 45 mol% for strain 131-8, and 32 mol% for *L. multipara* D32.

Pectinolytic enzymes. Dialyzed enzyme preparations from culture supernatants of all three strains produced reducing sugars from PGA at pH 8.5 but not at pH 5.5. The thiobarbituric acid assay was positive. This indicated that pectate lyases that formed unsaturated end products and had alkaline pH optima (32) were present. The addition of EDTA to the reaction mixture resulted in a 10-fold decrease in enzyme activity (Table 2). Addition of Ca²⁺ to a reaction mixture containing EDTA restored enzyme activity. These enzymes were similar to other pectate lyases in that calcium ions were required for optimal activity. The relative activities of the pectate lyases recovered from culture supernatants were greater when PGA was the substrate than when pectin was the substrate (Table 2).

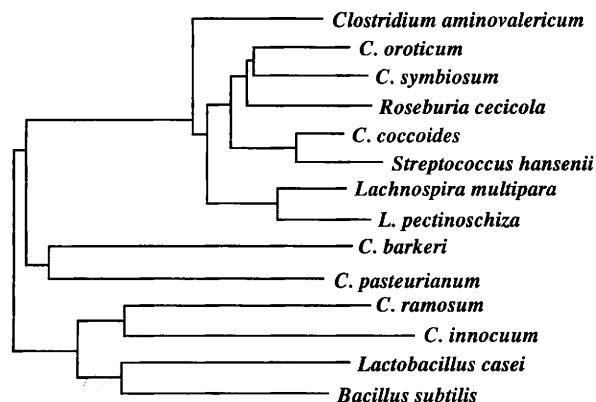


FIG. 3. Phylogenetic tree of *L. pectinoschiza* and other gram-positive bacteria based on 16S rRNA sequence comparisons. Bar = 0.05 estimated nucleotide change per position.

When strains were grown in medium PF containing pectin, production of pectin methylesterase was detected by the hydroxamic acid reaction (data not shown) and by the formation of methanol in culture supernatants.

Analysis of the end products obtained from PGA by thin-layer chromatography indicated that a dimer was the initial and major end product formed. After extended incubation (2 h), a trimer was also produced. The appearance of the dimer early in the reaction and the failure to find oligomers of different sizes as early products indicated that PGA was cleaved from the terminal end by an enzyme with an exo pattern of action.

16S rRNA sequence analysis. A similarity matrix showing the relationships among strain 150-1^T and 13 other gram-positive bacteria is shown in Table 3. A phylogenetic tree constructed from the data in Table 3 is shown in Fig. 3. The sequences in the assemblage marked by the divergence of *C. aminovalericum* constitute a monophyletic group (100% of the bootstrapped samples). Within this group, a monophyletic subset composed of strain 150-1^T and *L. multipara* was revealed by both evolutionary distance analysis and boot-

TABLE 3. 16S rRNA sequence similarity matrix for strain 150-1^T and other gram-positive bacteria

Species	% Similarity to:												
	<i>Bacillus subtilis</i>	<i>Lactobacillus casei</i>	<i>Clostridium innocuum</i>	<i>Clostridium ramosum</i>	<i>Clostridium aminovalericum</i>	<i>Lachnospira multipara</i>	<i>Lachnospira pectinoschiza</i>	<i>Streptococcus hansenii</i>	<i>Clostridium coccoides</i>	<i>Clostridium symbiosum</i>	<i>Clostridium oroticum</i>	<i>Roseburia cecicola</i>	<i>Clostridium pasteurianum</i>
<i>Lactobacillus casei</i>	86.7												
<i>Clostridium innocuum</i>	81.5	81.8											
<i>Clostridium ramosum</i>	81.9	82.1	84.0										
<i>Clostridium aminovalericum</i>	81.5	81.1	79.8	81.4									
<i>Lachnospira multipara</i>	79.2	79.0	78.8	79.0	90.0								
<i>Lachnospira pectinoschiza</i>	80.7	81.0	79.2	81.2	89.2	94.1							
<i>Streptococcus hansenii</i>	80.0	79.6	79.2	80.7	88.9	87.6	88.3						
<i>Clostridium coccoides</i>	81.0	81.4	79.3	81.2	89.5	88.4	90.2	94.1					
<i>Clostridium symbiosum</i>	81.0	81.4	79.6	80.5	90.1	89.4	89.6	89.7	91.2				
<i>Clostridium oroticum</i>	81.2	80.9	78.6	81.3	91.1	89.6	89.7	90.4	92.6	92.3			
<i>Roseburia cecicola</i>	80.4	80.8	77.4	80.7	88.9	90.5	91.4	89.4	91.1	91.1	91.3		
<i>Clostridium pasteurianum</i>	84.3	83.5	79.2	80.3	83.7	80.5	82.4	82.0	82.5	82.2	83.4	81.4	
<i>Clostridium barkeri</i>	83.9	81.0	79.7	79.7	82.5	80.0	80.7	81.6	81.5	80.3	82.0	79.7	84.0

TABLE 4. Phenotypic characteristics of *L. pectinoschiza* and *L. multipara*

Species	Major fatty acids	Cell width (μm)	Cell length (μm)	Temp range ($^{\circ}\text{C}$)	Flagella	Colony characteristics	G+C content (mol%)	Fermentation end products ^a
<i>L. pectinoschiza</i>	16:0, 14:0	0.4–0.6	2.4–3.1	30–45	Peritrichous	Opaque, umbonate	42–45	A, F, E, M, CO ₂
<i>L. multipara</i>	16:0, 16:0 ald ^b , 14:0	0.4–0.6	2.0–4.0	30–45	Monotrichous	Flat, filamentous	32	A, F, L, E, M, CO ₂ , H ₂

^a Fermentation end products in cultures grown in medium containing pectin. Abbreviations: A, acetate; F, formate; E, ethanol; M, methanol; L, lactate.

^b 16-carbon saturated aldehyde.

strapped parsimony analysis (the 16S rRNA sequences of *L. multipara* D32 and 40 were identical in the sequence region determined). A monophyletic group containing *C. coccoides* and *S. hansenii* was also identified. However, the relationships among other sequences in this assemblage (Fig. 3) were not significant as determined by bootstrap analysis or stable to altered sequence composition of distance trees. For example, the specific (monophyletic) relationship between *C. oroticum* and *C. symbiosum* should not be considered significant.

DISCUSSION

The pectinophilic bacteria that we isolated from the intestinal contents of pigs were present at densities of approximately 10^7 CFU/ml or 0.1% of the colonic population. Butine and Leedle (6) found populations containing 8×10^9 to 17×10^9 CFU of pectin-degrading bacteria per ml in the colonic contents of pigs. These counts were obtained by using a medium that contained Trypticase, yeast extract, depleted rumen fluid, and pectin (22). We obtained similar colony counts with medium PF; however, no pectinophiles were isolated from this medium.

We consider our isolates pectinophiles even though they have a somewhat less restrictive range of substrates than previously described pectinophiles. Our isolates grew much more rapidly on pectin and related compounds than on fructose, lactose, or cellobiose (doubling times of 55 min versus 240 to 600 min). The ability to utilize both lactose and cellobiose distinguishes these isolates from other pectinophiles and may serve as a survival mechanism when pectin is not present in the colon.

All three isolates produced at least two extracellular enzymes which degraded pectin, pectin methylesterase and exopeptidase lyase. *Bacteroides pectinophilus* and *Bacteroides galacturonicus*, two previously described pectinophiles (15), also produced extracellular pectin methylesterase and exopeptidase lyase. Both of the latter organisms cleaved the PGA polymer to unsaturated trimers. *Treponema pectinovorum*, a pectinophile present in human gingivae (38), formed extracellular pectin methylesterase and an endopeptidase lyase which broke PGA down to unsaturated dimers. Our strains did not produce any extracellular galacturonanase; this is characteristic of other pectinophiles as well.

Although spore-like structures were observed, there was no evidence of resistance to heating at 60 to 80°C or germination after exposure to 50% ethanol.

The 16S rRNA sequences of strain 150-1^T and *L. multipara* indicate that these organisms are members of a closely related monophyletic group. This suggests that these organisms were derived from an ancestor that presumably occupied a niche defined, in part, by pectin hydrolysis and/or utilization. *Lachnospira pectinoschiza* and *L. multipara* contain similar long-chain fatty acids (27), their cells are similar in size, and both organisms are mesophiles (4) (Table

4). *L. pectinoschiza* cells are peritrichous, form umbonate colonies, and have G+C contents of 42 to 45 mol%. *L. multipara* cells are monotrichous, form flat, filamentous colonies (4), and have a G+C content of 32 mol%. Both species produce acetate, formate, ethanol, methanol, and CO₂ when they are grown on media that contain pectin. *L. multipara* also forms lactate and H₂ (4). Devereux et al. (9) used the following formula to express the relationship between levels of rRNA similarity (*S*) and DNA relatedness (%DNA): $\log_{10} S = 0.0350 (\log_{10} \% \text{DNA}) - 0.0698$. When this formula was used, a DNA relatedness value of 17% was obtained for strain 150-1^T and *L. multipara*. However, this value is only an estimate, and the exact relationship between rRNA and genomic sequence divergence probably varies between groups of microorganisms (3). In keeping with the proposal to balance phenotypic information and genetic information in nomenclature (37), we recommend that our strains should be placed in the genus *Lachnospira*.

Description of *Lachnospira pectinoschiza* sp. nov. *Lachnospira pectinoschiza* (pec'ti.no.schiz.a. M.L. n. *pectinum*, pectin; Gr. adj. *schizon*, splitting; M.L. fem. *pectinoschiza*, pectin splitting [40]). Cells grown in medium PF broth are rod shaped, 0.36 to 0.56 μm wide, and 2.4 to 3.1 μm long and are arranged singly, in pairs, or in chains containing three to six cells. Cells peritrichous and motile by means of 6 to 18 flagella per cell. Gram positive.

Colonies on medium PF agar are opaque, circular with wavy edges, umbonate, and 3 to 5 mm in diameter. Growth in medium containing PGA is rapid, with a doubling time of approximately 55 min. Final cell yields are 2×10^9 to 3.5×10^9 CFU/ml. Growth occurs at temperatures ranging from 30 to 45°C. No growth occurs at 25 or 50°C.

Obligate anaerobes. Growth requires a fermentable carbohydrate such as pectin, PGA, gluconic acid, lactose, or cellobiose. Fructose is used after a lag period of 2 to 6 days. The following compounds do not support growth: L-arabinose, D-galactose, D-glucose, D-glucuronate, glycogen, inositol, inulin, D-maltose, D-mannitol, D-mannose, D-raffinose, L-rhamnose, D-ribose, salicin, D-sorbitol, soluble starch, sucrose, trehalose, D-xylose, amino acids, arabinogalactan, carboxymethyl cellulose, cellulose, gum arabic, pig gastric mucin, and xylan. Catalase is not produced. Nitrate is not reduced. Esculin and gelatin are not hydrolyzed. Growth occurs in medium PF broth supplemented with 20% bile.

The major end products of PGA fermentation are formate and acetate; minor amounts of ethanol and CO₂ are also formed. Methanol is formed when pectin is fermented.

Pectin is depolymerized by extracellular pectin methylesterase and a Ca²⁺-dependent exopeptidase lyase.

The G+C content is 42 mol%, as determined by thermal denaturation.

Isolated from cecal and colonic contents of pigs. The type strain, strain 150-1, has been deposited in the American Type Culture Collection as strain ATCC 49827.

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